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Instructions for use

Serazym®
Anti-Borrelia IgG

REF E-021

Σ **96**

CE

IVD

In-vitro-Diagnostic device

Enzyme immunoassay for detection of IgG antibodies to *Borrelia burgdorferi* in human serum samples

REF	Catalogue-No.	**LOT**	Lot-No.
	Storage temperature		Manufacturer
	Notice advices		Use by
		Σ	Number of determinations
	Consult Instructions for use		Biohazard

Introduction

Lyme borreliosis is a systemic infectious disease with various clinical manifestations.
The disease can be divided into 3 stages involving different organ systems. Cardinal symptoms of the first stage (4 to 8 weeks p. i.) are the erythema chronicum migrans and local or general lymphadenopathies (lymphadenosis cutis benigna). The clinical manifestations of the second stage (1 to 12 months p. i.) range from meningitis, meningopolyneuritis, encephalitis up to hemiparesis, myoneuralgia and arthralgia, especially knee joint arthritis. Cardiac manifestations like life-threatening myocarditis/pancarditis are rarely detected. The third stage (months to years p. i.) is characterized by chronic infection of the nervous system (neuroborreliosis, progressive encephalomyelitis), the skin (acrodermatitis chronica atrophicans) and the joints (chronic errosive arthritis). Especially late manifestations of Lyme borreliosis can reduce quality of life and antibiotic treatment is difficult. Therefore an early diagnosis of borrelia infections is of great importance.

The infectious agent of Lyme disease was isolated from ticks in 1982 by BURGDORFER et al. It belongs to spirochetes and represents the independent genus *Borrelia*.
The tick species *Ixodes ricinus* is the main vector of *Borrelia burgdorferi* in Europe. The occurrence of ticks within densely wooded rural areas correlates with the frequency of Lyme disease cases with a peak in the summer and autumn months. While the tick is taking blood from the host the borrelia are transmitted from the contents of the tick's intestine. Persons at risk for Borrelia infections are people who work in forestry or agriculture, hunters and campers, soldiers and all people that occasionally spend time in wooded areas.
The incidence of Lyme disease reaches from 2 to 40/100 000 in middle Germany to 300/100 000 in Austria. The estimated number of unrecognized cases is presumably higher, because a tick bite is not always recognized and the typical erythema chronicum migrans only develops in about 50 % of borrelia infections.

The direct detection of the infectious agent via culture from blood, cerebral spinal fluid (SCF) or skin biopsies undoubtedly represents the safest method for diagnosis of Lyme disease. Unfortunately this method is hampered by the long reproduction time of the borrelia, the complexity of the culture medium and the relatively low sensitivity in case of culture from patient material. Thus culture as well as borrelia detection from tissue sections via immunohistological methods are not suitable for routine diagnosis. Methods of choice are detection of IgG and/or IgM antibodies via immunofluorescence or enzyme imunoassay (ELISA). The ELISAs use borrelia sonicates, extracts, partially purified or recombinant antigens for plate coating. Because of the regionally differing occurrence of subtypes (genospecies) and the known variability of the cell surface proteins of *Borrelia burgdorferi,* antigen mixtures are often prefered. Confirmatory tests like western blot, dot blot or line assay are essential and the serological test results have to be interpreted together with the clinical picture. Negative test results do not exclude Lyme disease (early stage of infection, seronegative cases).

Literature:

1. Hauser, U., Lehnert, G., Lobentanzer, R., Wilske, B. Interpretation Criteria for Standardized Western Blots for Three European Species of Borrelia burgdorferi Sensu Lato J. Clin. Microbiol. 1997, 35: 1433-1444

2. Wilske, B.; Zöller, L.; Brade, V.; Eiffert, H.; Göbel, U.B.; Stanek, G.; Pfister, H.-W.
Lyme-Borreliose
MiQ Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik; 12
URBAN & FISCHER München Jena 2000
ISBN 3-437-41582-4

3. Kamradt, T., Krause, A., Priem, S., Burmester, G. R.
Die Lyme-Arthritis: Klinik, Diagnose und Therapie
Dt. Ärzteblatt 1998, 95: 214-219

4. Porstmann,T.
Labordiagnostik der Borrelieninfektion
in: Talaska, T. (Hrsg.): Symposium Lyme Borreliose: 35-38
DPC Akademie Bad Nauheim 1998

5. Talaska,T.
Diagnostische Methoden bei Borrelien-Infektionen - Übersicht - in: Talaska, T. (Hrsg.): Für die Praxis: Lyme-Borreliose
ISBN 3-00-002363-1

6. Tewald, F.,und Braun, R.
Durchführung und Interpretation serologischer Tests bei Verdacht auf Borrelieninfektion
Clin. Lab. 1998, 44: 897-902

7. Wilske, B., Fingerle, V., Hauser, U. und Rössler, D.
Borrelien
Diagnostische Bibliothek 48: 1-12
Blackwell Wissenschafts-Verlag, Juni 1997

8. Lawrenz, M. B., Hardham, J. M., Owens, R.T., Nowakowsky, J., Steere, A. C., Wormser, G.P., Norris, S. J.
Human Antibody Responses to VlsE Antigenic Variation Protein of Borrelia burgdorferi
J. Clin. Microbiol. 1999, 37/12: 3997-4004

9. Liang, T. F, Steere, A. C. , Marques A. R., Johnson, B. J. B., Miller J. N., Philipp M. T.
Sensitive and Specific Serodiagnosis of Lyme Disease by Enzyme-Linked Immunosorbent Assay with a Paptide Based on an Immunodominant Conserved Region of Borrelia burgdorferi VlsE
J. Clin. Microbiol. 1999, 37/12: 3990-3996

10. Peltomaa, M., McHugh, G., Steere, A. C.
The VlsE (IR_6) Peptide ELISA in the Serodiagnosis of Lyme Facial Paralysis
Otology & Neurology 2004, 25: 838-841

11. Schulte-Spechtel U., Lehnert G., Liegl G., Fingerle V., Heimerl C., Johnson B. J. B., Wilske, B.
Significant Improvment of the Recombinant Borrelia-Specific Immunoglobin G Immunoblot Test by Addition of VlsE and a DbpA Homologue Derived from Borrelia garinii for Diagnosis of Early Neuroborreliosis
J. Clin. Microbiol. 2003, 41/3: 1299-1303

Intended Use

The *Serazym*® Anti-Borrelia IgG is an in vitro diagnostic device for the detection of IgG antibodies against *Borrelia burgdorferi* in human serum or plasma samples.

Principle Of The Test

The *Serazym*® Anti-Borrelia IgG is an in vitro test developed for the quantitative determination of IgG antibodies against a mixture of antigens from *Borrelia afzelii* and *Borrelia garinii* supplemented with VsE in human serum.

The antibodies of the calibrators, positive control and diluted patient samples react with immobilized antigens on the solid phase of microtitration plates. Following an incubation period of 30 min at 37°C, unbound serum components are removed by washing the wells three times with wash buffer.

The bound antibodies react specifically with anti-human-IgG-F(ab)2 conjugated to horseradish peroxidase (HRP) within the incubation period of 30 min at 37°C. Excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colourless substrate solution of 3,3',5,5'-tetra-methylbenzidine (TMB) / hydrogen peroxide added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution (sulphuric acid) into the wells after 15 min at 37°C turning the solution from blue to yellow.

The absorbances read at 450/≥620 nm are directly proportional to the concentration of specifically bound antibodies.
A reference curve is created from the absorbances of the calibrators. The absorbances of the unknown serum samples are transformed into their corresponding antibody concentrations by reading from the reference curve.

Preparation And Storage Of Samples

Collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Lipaemic, haemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

Preparation before use

Allow samples to reach RT prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: Patient samples have to be diluted 1 : 101, e.g. 10 µl sample + 1000 µl sample diluent *(3)*, prior to assay.

The samples may be kept at 2…8 °C for up to two days. Long-term storage requires -20 °C.
(The calibrators and the controls are ready-to-use and should not be diluted).

Test Components For 96 Determinations

1 **WELLS**	**Microtitration plate,** 12 breakable 8 wells strips (total 96 individual wells) coated with antigens from *Borrelia afzelii, Borrelia garinii* and VlSE.	1 vacuum sealed with desiccant
2 **WASHBUF CONC 10X**	**Concentrated wash buffer** for 1000 ml solution	100 ml concentrate white cap
3 **DIL**	**Sample diluent**	100 ml ready to use black cap coloured red
4 **CAL 1 - 4**	**Calibrators 1 - 4** Contain Anti-*Borrelia*-IgG-Antibodies K1 = 40 U/ml K2 = 100 U/ml K3 = 250 U/ml K4 = 1000 U/ml	1.0 ml ready to use white cap coloured blue
5 **CONTROL -**	**Negative Control** diluted serum	1.0 ml ready to use green cap coloured blue
6 **CONTROL +**	**Positive Control** (diluted serum) Concentration see leaflet enclosed	1.0 ml ready to use red cap coloured blue
7 **CONJ HRP**	**Conjugate** Containing anti-human-IgG-F(ab)2 (sheep) coupled with HRP	15 ml ready to use red cap coloured red
8 **SUBSTR TMB**	**Substrate** 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide	15 ml ready to use blue cap
9 **STOP**	**Stop solution** 0.25 M sulphuric acid	15 ml ready to use yellow cap

Materials Required But Not Provided

- Adjustable one channel micropipette 0.100 – 1.000 ml and 0.010 – 0.100 ml
- Adjustable 8-channel micropipette 0.050 – 0.200 ml
- Pipette tips
- Graduated measuring flasks 10 ml and 100 ml
- Microtitration plate washer (automatic or hand wash head)
- Microtitration plate reader with 450 nm filter for measurement and ≥ 620 nm filter for reference
- Distilled or de-ionized water
- Test tubes (2 ml) for sample dilution

Preparation And Storage Of Reagents

Kit size and expiry

The *Serazym*® Anti-Borrelia IgG has been designed for 96 determinations.

The complete kit with unopened reagent bottles and microtitration strips is stable until the expiry date printed on the kit box in case of storage at 2…8 °C. Once opened all kit components are stable for up to 2 months under appropriate storage conditions (2…8 °C). When stored at 2…8 °C the diluted ready-to-use wash solution is stable for up to 1 month.

Reagent preparation

Allow all components to reach RT prior to use in the assay.

The microtitration plate is vacuum sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the **WASHBUF CONC 10X** ***(2)*** 10 times (1 + 9) with de-ionized or distilled water.
For example: 10 ml **WASHBUF CONC 10X** ***(2)*** + 90 ml distilled water.

Avoid exposure of the TMB substrate solution to light!

Assay Procedure

- **Dilute patient sera with sample diluent *(3)* 1 + 100 (v/v) e.g. 10 µl serum + 1000 µl sample diluent *(3)***
- **Avoid any time shift during dispensing of reagents and samples.**
- **Make sure the soaking time of the wash solution in the wells is at least 5 seconds per wash cycle and that the remaining fluid is completely drained in every wash cycle.**

Working steps

1. Warm all reagents to RT before use. Mix gently without causing foam.
2. Dispense
   100 µl *CAL 1 - 4* (ready-to-use calibrators 1, 2, 3, 4) ***(4)***,
   100 µl *CONTROL-* (ready-to-use control) ***(5)***
   100 µl *CONTROL+* (ready-to-use control) ***(6)*** and
   100 µl diluted serum samples resp.
   into the intended wells.
3. Cover plate, incubate **30 min** at 37°C.
4. Decant, then wash wells **three** times using **300 µl** wash solution (made of ***(2)***).
5. Add **100 µl *CONJ HRP (6)*** to each well.
6. Cover plate, incubate **30 min** at 37°C.
7. Decant, then wash wells **three** times using **300 µl** wash solution (made of ***(2)***).
8. Add **100 µl *SUBSTR TMB (7)*** to each well.
9. Incubate **15 min** at 37°C protected from light.
10. Add **100 µl *STOP (8)*** to each well and mix gently.
    Read absorbances at **450 nm** / **≥ 620 nm** within 30 min after reaction stop.

Result Interpretation

Create a reference curve by plotting the mean absorbances of the calibrators *CAL 1-4* (Y-axis) to their corresponding antibody-concentrations (X-axis). The stated calibrator concentrations already imply the regular dilution factor of 101.
Determine the antibody-concentrations of the unknown samples by referring their mean absorbances to the reference curve. Samples with absorbances exceeding calibrator *CAL 4* should be retested in higher dilutions; in this case the additional dilution factor has to be considered for final result determination (e.g. using a sample dilution of 1:200 the results have to be multiplied with two).

Serum samples with calculated antibody concentrations > 61U/ml have to be declared as positive for anti *Borrelia burgdorferi* IgG antibodies, serum samples with concentrations < 50 U/ml have to be declared as negative for *Borrelia burgdorferi* IgG antibodies. Serum samples with calculated antibody concentrations between 50 and 61 U/ml should be retested. However, analysis of a new serum sample collected from the same patient 1-2 weeks later is recommended.

Reference Values

***Serazym*® Anti-Borrelia-IgG**	
Negative	**< 50 U/ml**
Positive	**> 61 U/ml**
Borderline	**50 - 61 U/ml**

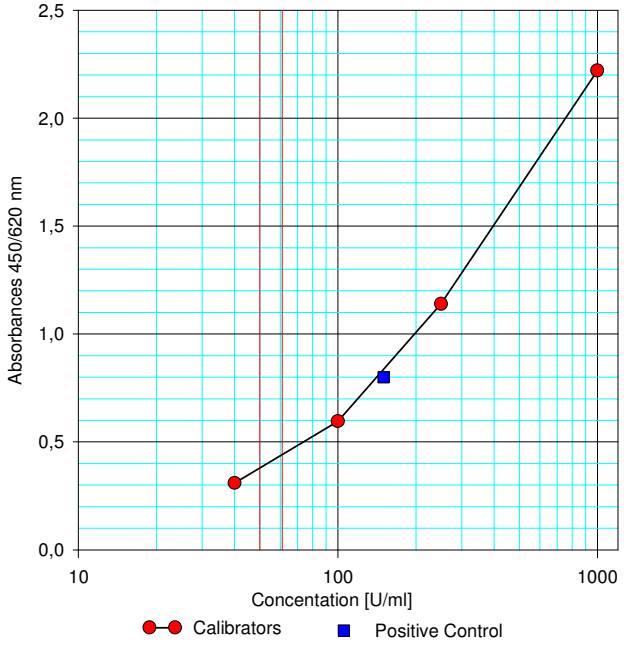
Test validity

The test run is valid if:

- absorbance of calibrator *CAL 1* ≤ 0.50
- absorbance of calibrator *CAL 4* ≥ 1.50

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Typical reference curve



Limitations of the procedure

The early antibodies during Borrelia infection are characterized by their specific reactivity to the flagellin protein as the main target. The internal sequence of this protein is specific for *Borrelia burgdorferi* but there are sequence homologies to other spirochetes in the regions C-terminus and N- terminus, respectively. In certain cases of infections with other spirochetes this may create false positive results due to cross reactivity of the antibodies.
Therefore, reactive serum samples should be verified by a confirmatory test (e.g. Line-Assay or Westernblot).

The result interpretation should always consider clinical findings. Individual cases may require repeated investigations of samples taken at intervals of several weeks.

As in other immunoassays, impurities and cross contamination of reagents and samples by fungi and bacteria can produce false positive as well as false negative results.

Incorrect dilutions of samples, incorrect washing of the strips and also incorrect timing can produce erroneous results.

Performance Characteristics

Precision

Intra-assay coefficient of variation (CV) in the *Serazym*® Anti-Borrelia IgG

Sample	Concentration U/ml	CV (%)
1	842	8,1
2	424	5,7
3	156	7,3
4	104	6,5

Inter-Assay-Variationskoeffizienten (VK):

Sample	Concentration U/ml	CV (%)
1	611	6,9
2	349	6,9
3	147	7,9
4	70	6,0

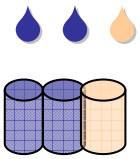
Diagnostic specificity and sensitivity

A population of 3000 healthy blood donors was investigated to determine the specificity of *Serazym*® Anti-Borrelia-IgG as well as serum samples from 70 patients suffering from Borreliosis for determination of sensitivity.

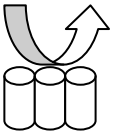
According the results *Serazym*® Anti-Borrelia-IgG has a specificity of 98 % and a sensitivity of 95 %.

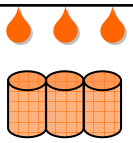
Incubation Scheme

Serazym® Anti-Borrelia IgG (E-021)

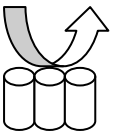


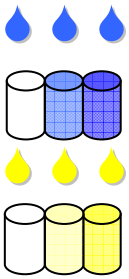
100 µl	***CAL 1 - 4 (4)***
100 µl	***CONTROL- (5)* and**
100 µl	***CONTROL+ (6)* and**
100 µl	**diluted serum samples resp.**
30 min	incubation at 37 °C
3 x Wash	with wash solution





100 µl	***CONJ HRP (7)***
30 min	incubation at 37 °C
3 x wash	with wash solution





100 µl	***SUBSTR TMB (8)***
15 min	incubation at 37 °C protected from light
100 µl	***STOP (9)***
Read absorbances	at 450/ ≥ 620 nm

Common Advices and Precautions

This kit is for *in-vitro* use only. Follow the working instructions carefully. The kit should be performed by trained technical staff only.

The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

Do not use or mix reagents from different lots or reagents from other manufacturers.

Avoid time shift during dispensing of reagents.

All reagents should be kept at 2…8 °C before use.

Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucous membranes.

Handle all components and all patient samples as if potentially hazardous.

Since the kit contains potentially hazardous materials, the following precautions should generally be observed:

- Do not smoke, eat or drink while handling kit material,
- Always use protective gloves,
- Never pipette material by mouth,
- Note safety precautions of the single test components.